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TECHNICAL MANUSCRIPT 479

IMPROVED GROWTH OF MAMMALIAN AND INSECT CELLS IN CULTURE WITH HIGH LEVELS OF CHOLINE

Stanley C. Nagle, Jr.

SEPTEMBER 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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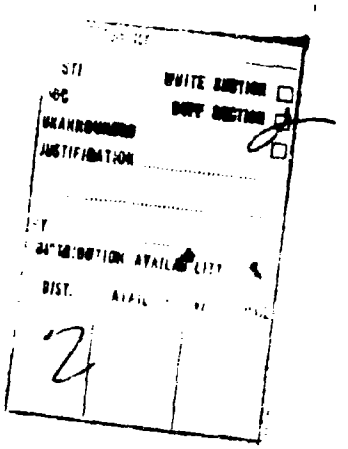

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Stanley C. Nagle, Jr.

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B52?30LA082

September 1968

IMPROVED GROWTH OF MAMMALIAN AND INSECT CELLS IN CULTURE WITH HIGH LEVELS OF CHOLINE

ABSTRACT

Relatively high concentrations of choline chloride produce improved growth of HeLa, L, and Aedes aegypti cells in tissue culture. Approximately 50 mg per liter is optimal for both mammalian and insect cell lines.

Most cell growth media contain about 1 mg per liter of choline, consistent with concentrations usually employed for vitamins. One notable exception is the medium described by Waymouth* containing 250 mg per liter. I observed that, for L cells growing in a chemically defined medium, increased choline permitted continuing growth of cells in the absence of medium replenishment over a longer period of time. Choline levels became deficient, apparently, when it was used at 1 mg per liter. This observation was tested further using HeLa (human), L (mouse), and Aedes aegypti (mosquito) cells.

The mammalian cells were grown in suspension in a heat-stable chemically defined medium as described by Nagle.**

Mosquito cells were grown in suspension in a medium modified from that described earlier.*** The insect cell medium, designated #35, contained the following components in milligrams per liter: L-alanine, 100; L-arginine HCl, 400; L-aspartic acid, 300; L-cysteine HCl, 100; L-glutamic acid, 400; glycine, 400; L-histidine HCl, 200; L-isoleucine, 150; L-leucine, 300; L-lysine HCl, 300; L-methionine, 60; L-phenylalanine, 150; L-proline, 300; L-serine, 150; L-threonine, 75; L-tryptophan, 50; L-tyrosine, 100; L-valine, 150; NaCl, 4,500; KCl, 2,500; $CaCl_2 \cdot 2H_2O$, 400; $MgCl_2 \cdot 6H_2O$, 400; $MgSO_4 \cdot 7H_2O$, 400; $NaH_2PO_4 \cdot H_2O$, 200; glucose, 5,000; D-biotin, 1.0; choline Cl, 1.0 (except where noted); folic acid, 1.0; niacinamide, 1.0; Ca pantothenate, 2.0; pyridoxal HCl, 1.0; thiamine HCl, 1.0; i-inositol, 1.0; riboflavin, 0.1; vitamin B_{12}, 0.002. The medium was adjusted to

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* Waymouth, C. 1959. Rapid proliferation of sublines of NCTC clone 929 (strain L) mouse cells in a simple chemically defined medium (MB 752/1). J. Nat. Cancer Inst. 22:1003-1017.

** Nagle, S.C., Jr. 1968. Heat-stable chemically defined medium for growth of animal cells in suspension. Appl. Microbiol. 16:53-55.

*** Nagle, S.C., Jr.; Crothers, W.C.; Hall, N.L. 1967. Growth of moth cells in suspension in hemolymph-free medium. Appl. Microbiol. 15:1497-1498.

pH 6.5 with KOH and sterilized by autoclaving at 121 C for 15 minutes. After cooling, 15 centipoises methylcellulose (as a 2% sterile solution) was added to a final concentration of 0.05%, L-glutamine was added (500 mg per liter) as a dry powder after autoclaving in a stoppered 15-ml serum bottle for 1 hour at 121 C, heated fetal bovine serum (15 minutes at 56 C) was added to a final concentration of 5%, and streptomycin and penicillin were added at final concentrations of 100 µg and 100 units per ml, respectively.

Cultures of HeLa and L cells were grown at 34 to 36 C in rubber-stoppered 100-ml serum bottles, containing 25 ml of medium, on a New Brunswick Gyrotory shaker* operating at 124 to 130 rpm. Mosquito cells were grown at 26 to 28 C in 250-ml Falcon plastic flasks,** also containing 25 ml of medium, on a New Brunswick Gyrotory shaker operating at 60 rpm. Cell numbers were determined by hemacytometer count. Viability of mammalian cells was routinely observed by counting only those cells with the ability to exclude trypan blue. The trypan blue procedure was not used for mosquito cells; only obviously healthy, intact cells were counted, however.

Results of growth of the three cell lines in their respective media containing graded levels of choline are given in Table 1. Three cultures were prepared for each concentration of choline for each cell line. The data represent the average values for at least two sets of triplicate cultures for each concentration, based on the highest counts obtained in 5, 6, and 7 days of incubation for HeLa, L, and mosquito cells, respectively. Media were not changed during growth. Fifty mg per liter of choline chloride appeared to be optimal for all cells. Cell populations in media with 50 mg per liter compared with those obtained with 1 mg per liter were higher by 28, 143 and 57%, respectively, for HeLa, L, and A. aegypti cells. Growth of A. aegypti cells in the absence of added choline is explained by the fact that the growth medium for these cells contained 5% serum.

Daily replacement of media with fresh media containing 50 mg of choline per liter resulted in a yield of 32.7×10^5 HeLa cells per ml in 9 days and a yield of 59.8×10^5 L cells per ml in 8 days. These values are about equal to (HeLa) or better than (L) populations reported earlier*** and were obtained 1 day (HeLa) or 2 days (L) sooner. A. aegypti cells reached populations of only 10.6×10^5 per ml in 9 days under these conditions. Comparison of these figures with data given in Table 1 shows that the chemically defined medium for HeLa and L cells may become depleted of nutrients when not replenished or that growth is limited by accumulation of certain inhibitory metabolites. Limitation of growth of A. aegypti cells probably was not due to depletion of nutrients because medium changes were not beneficial.

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* New Brunswick Scientific Co., Inc., New Brunswick, N.J. 08903.

** Falcon Plastics Division of B-D Laboratories, Inc., Los Angeles, Calif. 90045.

*** Nagle, S.C., Jr. 1968 Heat-stable chemically defined medium for growth of animal cells in suspension. Appl. Microbiol. 16:53-55.

TABLE 1. EFFECTS OF CHOLINE ON GROWTH OF HeLa, L, AND AEDES AEGYPTI CELLS

Choline Cl Concn, mg/liter	No. of Cells per ml, x 10^5[a]		
	HeLa	L	A. aegypti
0	2.2	3.0	9.7
1	6.1	6.1	10.9
10	6.7	11.4	15.8
50	7.8	14.8	17.1
100	6.1	14.7	16.0
200	5.0	14.1	12.1

a. Zero-time populations were 2 x 10^5 to 3 x 10^5 per ml for all cell types.

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14. Key Words

Choline chloride
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